H I L G A R D I A

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APHID TRANSMISSION OF MILD MOSAIC VIRUS OF ANNUAL STOCK[1]

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SUMMARY

Five species of aphids, tested in lots of 20, were demonstrated to be vectors of mild-mosaic virus of annual stock; these are:

Bur clover or cowpea aphid, *Aphis medicaginis* Koch
Cabbage aphid, *Brevicoryne brassicae* Linnaeus
Artichoke aphid, *Myzus braggi* (Gillette)
Green peach aphid, *Myzus persicae* (Sulzer)
Turnip or false cabbage aphid, *Rhopalosiphum pseudobrassicae* (Davis)

Of these, only the turnip aphid breeds on annual stock plants under natural conditions. It causes pale-green circular areas around the mouth-part punctures, and, when abundant, dwarfing and yellowing or blanching of the flowers.

The turnip aphid failed to transmit the virus to nine varieties of healthy cauliflower.

Several infections were obtained with single turnip and green peach aphids fasted for 2 hours, fed ½, 1, 2, 5, or 10 minutes on leaves from infected stock plants, and then transferred to healthy plants, 1 aphid per plant. No infections were obtained with several hundred turnip, green peach, and cabbage aphids tested singly without fasting and with longer periods on infected stock plants.

In tests on retention of the virus, turnip aphids, fasted for 30 minutes, then fed singly 5 or 10 minutes on mild-mosaic-infected annual stock and 5 or 10 minutes on 5 or 6 successive healthy stock plants, produced infections only in the first healthy plant.

Lots of 20 turnip aphids had lost their infectivity by the fourth day after transfer from an infected to a previously healthy stock plant. If allowed to remain on the plant from 7 to 13 days, however, the aphids were able to recover the virus from the plant they had inoculated; this was long before symptoms appeared. The incubation period of the disease in the original inoculated plants varied from 16 to 22 days.

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INTRODUCTION

In 1930, commercial growers of annual stock (*Mathiola incana* var. *annua*) observed a serious disease that caused breaking in the color of flower petals. Field infection was severe from 1931 to 1948. The disease destroyed the value of the plants for cut flowers and reduced seed production. The trouble was identified as a virus disease (Tompkins, 1934).[4] Tompkins (1939) named it mild mosaic of annual stock, and distinguished it from severe mosaic, another virus disease of this host, which causes even more severe breaking and occurs under field conditions in San Pablo, California. He described the symptomatology, transmission, host range, and properties of the two viruses.

Breaking in the color of petals has been reported to result from mechanical inoculation with several crucifer mosaic viruses, in addition to the two stock viruses—namely, turnip mosaic (Tompkins, 1938; Chamberlain, 1939), cabbage black ring (Tompkins, Gardner, and Thomas, 1938), Chinese cabbage mosaic (Tompkins and Thomas, 1938), cabbage mosaic (Larson and Walker, 1938), and horseradish mosaic (Tompkins, 1939). Investigators in various countries have reported breaking in the color of petals or mosaic disease in annual stock, without identifying the causal virus (see Tompkins, 1939).

Annual stock has been demonstrated to be naturally infected with sugar-beet curly top in California (Severin, 1934), and with an unidentified disease resembling California aster yellows; but these do not cause breaking.

In connection with the investigation of other phases of mild and severe mosaics of annual stock by Tompkins (1939), experiments were undertaken in 1934 on various phases of aphid transmission. Tompkins reported early results obtained by the senior author: the turnip or false cabbage aphid, *Rhopalosiphum pseudobrassicae* (Davis), the cabbage aphid, *Brevicoryne brassicae* Linnaeus, and the green peach aphid, *Myzus persicae* (Sulzer), were demonstrated to be vectors of both viruses. The turnip aphid breeds on stock under natural conditions, the other two do not.

The present paper reports further results of experiments on aphid transmission of mild mosaic of annual stock; phases investigated include transmission of the virus by several aphid species from infected stock plants to healthy stock and cauliflower plants, comparison of transmission of the virus by mechanical inoculation with that by three species of aphids, transmission of the virus by single aphids and during short feeding periods, the retention of the virus, and loss and recovery of infectivity by aphids. Symptoms on foliage and flowers caused by feeding of noninfective aphids were studied and differentiated from those caused by the mild-mosaic virus.

MATERIALS AND METHODS

The virus causing mild mosaic of annual stock was obtained at Montara, San Mateo County. Plants of the Fiery Blood Red variety of annual stock and the February variety of cauliflower grown from seeds were used in all experiments. Methods used in aphid-transmission experiments were similar to those used previously (Severin and Freitag, 1938). The carborundum method (Rawlins and Tompkins, 1936) was used in mechanical inoculations.

[4] See Literature Cited for citations, referred to in the text by author and date.

DISTRIBUTION

Annual stock naturally infected with mild mosaic is generally distributed on seed farms and in home gardens in the coastal districts of California. Stock plants showing breaking in the color of the petals were obtained at Davis and the virus was recovered and transferred by means of the turnip aphid, *Rhopalosiphum pseudobrassicae* (Davis) (plate 1), to healthy stock.

SYMPTOMATOLOGY

Induced by Feeding of Aphids. Noninfective and infective turnip aphids, *Rhopalosiphum pseudobrassicae,* produced pale-green circular areas (plate 2, *B*) around the mouth-part punctures on the leaves. The flowers of stock plants which had large populations of aphids (plate 1) were dwarfed, and frequently the tips of the petals were yellow or white; sometimes most or all of the petals were yellowish green (plate 2, *C*).

Mild Mosaic. Tompkins (1934, 1939) has described the symptoms of mild mosaic on annual stocks. The more important symptoms are briefly reviewed here for comparison with the symptoms induced by the feeding of aphids.

The first symptom of mild mosaic on the younger leaves of annual stock appears 2 to 3 weeks after inoculation, as a clearing of the veins and veinlets (plate 3, *B, C, D*) followed by mottling consisting of pale and dark-green areas (plate 3, *E*). These symptoms rarely occur on naturally infected stock plants. Sometimes the apical leaves are distorted, curled, and puckered. Infected plants are slightly or severely stunted with shortened internodes.

A striking flower symptom is breaking in color of the petals (plate 4, *B, C*). Sometimes the cluster of flowers at the apical end of the stems is apparently normal, while the lower racemes show breaking; usually, however, all racemes show breaking. A reduction of the number and size of the seed pods occurs.

APHID TRANSMISSION OF VIRUS

By Vectors That Do Not Breed on Stock under Natural Conditions. No intensive investigations were undertaken on aphid vectors which do not breed on annual stock plants under natural conditions.

Bur clover or cowpea aphids, *Aphis medicaginis* Koch, collected on lamb's-quarters (white pigweed) (*Chenopodium album*) and on rough pigweed (*Amaranthus retroflexus*) growing among annual stock plants near Montara, were transferred from these weeds to stock infected with mild mosaic. A high mortality of the aphids occurred on annual stock. Three of 6 lots of 20 aphids each, changed from mosaic to healthy annual stock plants, caused infection.

Artichoke aphids, *Myzus braggi* (Gillette), collected on artichokes near El Granada, were transferred to mosaic-infected annual stock, and then to healthy annual stock plants. Three lots of 20 aphids produced 2 infections.

Green peach aphids, *Myzus persicae*, collected on sugar beets, were transferred to naturally infected stock plants and thence to healthy stock and cauliflower plants. The results are given in table 1. The cabbage aphid, *Brevicoryne brassicae*, was also demonstrated to be a vector of this virus.

By a Vector That Breeds on Stock under Natural Conditions. Whenever a large population of the turnip aphid was found on naturally infected

annual stock plants showing breaking in the flowers, lots of 20 aphids were transferred from the mosaic to healthy annual stock plants and to healthy cauliflower seedlings. This aphid transmitted the virus to 60 per cent of the stock plants inoculated, but to none of the cauliflower plants (table 1).

From Experimentally Infected to Healthy Annual Stock Plants and Varieties of Cauliflower. Lots of 20 infective turnip aphids were transferred from

TABLE 1

TRANSMISSION OF MILD-MOSAIC VIRUS FROM NATURALLY INFECTED TO HEALTHY ANNUAL STOCK BY TWO SPECIES OF APHIDS

Aphid species and district in which infected plants were found	Annual stock		Cauliflower	
	Inoculated	Infected	Inoculated	Infected
Turnip aphid, *Rhopalosiphum pseudobrassicae:*				
Alameda County:				
Berkeley	10	2	10	0
Berkeley	6	5	6	0
San Francisco County:				
San Francisco	6	2	6	0
San Mateo County:				
Burlingame	12	10	12	0
Montara	10	8	10	0
Montara	10	8	10	0
Montara	10	3	10	0
Yolo County:				
Davis	12	6	12	0
Davis	6	5	6	0
Total	82	49	82	0
Percentage	..	*60*	..	*0*
Green peach aphid, *Myzus persicae:*				
Alameda County:				
Berkeley	5	4	5	0
Berkeley	5	3	5	0
San Francisco County:				
San Francisco	5	3	5	0
San Mateo County:				
Burlingame	5	2	5	0
Montara	5	2	5	0
Montara	5	2	5	0
Montara	5	2	5	0
Yolo County:				
Davis	5	1	5	0
Davis	5	1	5	0
Total	45	20	45	0
Percentage	..	*45*	..	*0*

experimentally infected annual stock to healthy annual stock plants and nine varieties of cauliflower. Table 2 shows that 51 per cent of the annual stock plants, but none of the cauliflower plants, were infected. Each cauliflower plant was again inoculated with a lot of 20 infective false cabbage aphids, and again no infections occurred. The fact that no infection of cauliflower was obtained with the mild-mosaic virus of annual stock serves to differentiate this virus from the cauliflower-mosaic virus, a conclusion which Tompkins (1937) reached from his results with mechanical inoculation.

Comparison of Aphid Transmission and Mechanical Inoculation. The transmission of the virus from experimentally infected to healthy stock plants by mechanical inoculation was compared with transmissions by three species of aphids. The virus extract from infected plants upon which the aphids had

TABLE 2

TRANSMISSION OF VIRUS FROM STOCKS EXPERIMENTALLY INFECTED WITH MILD MOSAIC TO HEALTHY ANNUAL STOCK AND CAULIFLOWER BY THE TURNIP APHID

Mosaic-infected stock plant no.	Annual stock		Cauliflower		
	Inoculated	Infected	Variety	Inoculated	Infected
1	5	3	Danish Perfection	5	0
2	5	0	Dryweather Danish Giant	5	0
3	5	1	Extra Early Dwarf Erfurt	5	0
4	5	2	Hartmans Special	5	0
5	5	1	Hartmans Special Medium	5	0
6	5	1	Late Pearl	5	0
7	5	4	February 759*	5	0
8	5	5	Early March 713*	5	0
9	5	5	Mission Special 4577*	5	0
Total	45	22		45	0
Percentage	..	*49*		..	*0*

* Grown from seed from Ferry-Morse Seed Co., San Francisco.

TABLE 3

COMPARISON OF TRANSMISSION OF MILD-MOSAIC VIRUS OF ANNUAL STOCK BY MECHANICAL INOCULATION AND BY THREE SPECIES OF APHIDS

Number of plants from which virus was recovered	Mechanical inoculation			Aphid transmission			
	Plants inoculated	Plants infected	Per cent infected	Aphid species	Plants inoculated	Plants infected	Per cent infected
5	25	20	80	Cabbage aphid, *Brevicornye brassicae*	25	1	4
10	50	7	14	Turnip aphid, *Rhopalosiphum pseudobrassicae*	50	22	44
5	25	15	60	Green peach aphid, *Myzus persicae*	25	13	52

fed was inoculated into healthy plants. The results obtained are given in table 3. The turnip aphid was more efficient in transmitting the virus than was mechanical inoculation, the cabbage and green peach aphids less so.

By Single Aphids. An attempt was made to determine the efficiency of virus transmission by three species of mature aphids fed 1 day on mosaic stock plants, then each fed singly on a healthy stock plant. Equal numbers of winged and wingless aphids were used. Not a single infection was obtained with 300 turnip aphids, 200 cabbage aphids, and 150 green peach aphids.

TABLE 4

SHORT FEEDING TIME ON MILD-MOSAIC STOCK PLANTS BY SINGLE WINGLESS APHIDS OF TWO SPECIES

Aphid species	Feeding time, minutes									
	0.5		1		2		5		10	
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected
Turnip aphid, *Rhopalosiphum pseudobrassicae*	5	1	5	0	5	0	5	1	5	0
Green peach aphid, *Myzus persicae*	5	2	5	1	5	1	5	0	5	1

TABLE 5

RETENTION OF MILD-MOSAIC VIRUS OF ANNUAL STOCKS BY TWO SPECIES OF APHIDS

Aphid species and test no.	Number of aphids on first plant	Number of annual stock					
		Inoculated 1st day	Infected 1st day	Inoculated 2d day	Infected 2d day	Inoculated 3d day	Infected 3d day
Turnip aphid, *Rhopalosiphum pseudobrassicae:*							
Test 1	5	12	2	12	0	12	0
Test 2	10	6	1	6	0	6	0
Test 3	20	7	4	7	0	7	0
Test 4	20	6	3	6	0	6	0
Test 5	20	6	1	6	0	6	0
Test 6	20	5	4	5	0	5	0
Test 7	20	5	1	5	0	5	0
Test 8	20	5	1	5	0	5	0
Test 9	20	4	1	4	0	4	0
Total	..	56	18	56	0	56	0
Percentage	..	..	*32*	..	*0*	..	*0*
Green peach aphid, *Myzus persicae:*							
Test 1	20	5	4	5	0	5	0
Test 2	20	5	3	5	0	5	0
Test 3	20	5	3	5	0	5	0
Test 4	20	5	3	5	0	5	0
Test 5	20	5	2	5	0	5	0
Test 6	20	5	2	5	0	5	0
Test 7	20	5	2	5	0	5	0
Test 8	20	5	2	5	0	5	0
Test 9	20	5	2	5	0	5	0
Test 10	20	5	2	5	0	5	0
Test 11	20	5	1	5	0	5	0
Test 12	20	5	1	5	0	5	0
Test 13	20	5	1	5	0	5	0
Test 14	20	5	1	5	0	5	0
Total	..	70	29	70	0	70	0
Percentage	..	..	*41*	..	*0*	..	*0*

Because of the failure of the single-aphid tests, further tests of the turnip and green peach aphids were made with a different technique. Noninfective, wingless aphids of these two species were fasted in a phial for 2 hours. Five lots of 5 aphids each were transferred from the phial to a leaf from an infected stock plant and fed ½, 1, 2, 5, or 10 minutes; and then each aphid was transferred to a healthy plant. With the turnip aphid two infections were obtained, at ½- and 5-minute feeding times, and with the green peach aphid five infections at ½-, 1-, 2-, and 10-minute feeding times (table 4).

TABLE 6

RETENTION OF MILD-MOSAIC VIRUS OF ANNUAL STOCK BY SINGLE TURNIP APHIDS, IN SHORT FEEDING PERIODS

Time on diseased annual stock, minutes	Penetration time of stylets in first healthy annual stock, minutes	Feeding time on successive healthy annual stock, minutes*					
		5	10	10	10	10	10
5	7	+	−	−	−	−	−
5	5	+	−	−	−	−	−
10	4	..	+	−	−	−	−
10	3	..	+	−	−	−	−
10	2	..	+	−	−	−	−
Total	4.2	2+	3+	5−	5−	5−	5−

* The plus sign (+) indicates the production of the disease, and the minus sign (−) shows that no disease resulted.

Effect on Flowers. Tests were made to determine the effect on the flowers of infecting annual stock plants at various stages of flower-bud development; the turnip aphid was used for transmission. On plants infected when flower buds were large, the apical cluster of flowers sometimes showed no breaking in color of the petals, but the lower flowers developed breaking. Plants infected before flower buds were visible or when buds were small showed breaking in all flowers.

RETENTION OF VIRUS

By Varying Numbers of Aphids. The retention of the virus was determined for the turnip and the green peach aphids. In the preliminary work, lots of 5 and 11 aphids were transferred daily for 20 days to successive healthy plants, and the aphids remained on the last healthy annual stock plant for one week. No infections were obtained after the first day.

In later work (table 5) lots of 20 infective aphids reared on mild-mosaic stock plants were transferred daily for 3 days to successive healthy stock plants. Both species of aphids transmitted the virus from diseased to healthy annual stock during the first day, but none of the lots tested caused infection the second or third days.

By Single Aphids in Short Feeding Times. Noninfective, mature, wingless, turnip aphids were fasted for 30 minutes in a moist chamber, then were transferred singly to annual stock infected with mild mosaic for a feeding time of 5 or 10 minutes, and then to 5 or 6 successive healthy annual stocks for a feeding time of 5 or 10 minutes on each plant. Table 6 shows that 5 aphids tested singly transmitted the virus to only the first annual stock plant. Sixty-six aphids failed to transmit the virus (not included in table 6).

LOSS AND RECOVERY OF INFECTIVITY BY TURNIP APHIDS

An experiment was conducted to determine whether the turnip aphid could recover the mild-mosaic virus from annual stock plants before breaking in color of petals appeared. A large population of aphids reared on mild-mosaic-

TABLE 7

LOSS AND RECOVERY OF INFECTIVITY BY TURNIP APHID ON STOCK PLANTS INOCULATED WITH MILD-MOSAIC VIRUS

Original plant number	Results* when a lot of 20 aphids was transferred from the first inoculated plant to a second healthy plant on:											Days to color breaking on petals of original plants
	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13th day	14th day	
1	−	−	−	−	+	−	−	+	−	+	+	37
2	−	−	−	−	+	−	−	−	−	+	+	38
3	−	−	−	+	−	−	+	+	−	−	+	40
4	−	−	−	−	−	−	−	−	+	−	+	37
5	−	−	−	−	−	−	+	−	−	+	+	44
6	−	−	−	−	+	−	−	−	−	+	+	44
7	−	−	−	−	−	+	−	−	−	−	+	44
8	−	−	−	−	+	−	+	−	−	−	+	46
9	−	−	−	−	−	−	−	+	−	+	−	38
10	−	−	−	−	+	−	−	−	−	+	+	40
11	−	−	−	−	−	+	−	−	+	+	−	39
12	−	−	−	−	+	−	−	−	−	+	−	45
13	−	−	−	+	−	−	−	+	+	−	−	34
14	−	−	−	−	−	−	+	+	+	−	−	38
15	−	−	−	−	−	−	−	−	+	+	−	38
16	−	−	−	−	+	−	−	−	−	−	+	39
17	−	−	−	−	−	−	−	−	−	+	−	40
18	−	−	−	−	−	−	−	−	+	−	+	41
19	−	−	−	−	−	−	+	−	+	−	−	46
20	−	−	−	−	+	−	+	+	−	−	−	39
21	−	−	−	+	−	−	−	+	−	−	−	30
22	−	−	−	−	−	+	−	−	−	−	−	42
23	−	−	−	−	−	−	−	−	+	−	+	41
24	−	−	−	−	+	+	−	−	−	−	+	43
25	−	−	−	−	+	+	−	−	−	−	+	44
26	−	−	−	−	+	+	−	−	−	−	−	40
27	−	−	−	−	−	+	−	−	−	+	+	43
28	−	−	−	−	−	+	−	−	−	−	−	44
29	−	−	−	−	−	+	+	−	−	−	+	47
30	−	−	−	−	−	+	+	−	−	+	+	48
Total +	0	0	0	3	11	10	8	7	8	12	17	
Total −	30	30	30	27	19	20	22	23	22	18	13	

* The plus sign (+) indicates the production of the disease, and the minus sign (−) shows that no disease resulted.

infected stock plants was transferred to 36 large healthy stock plants for 3 days. Daily from the fourth to the fourteenth day, one lot of 20 of these aphids was transferred from each of the plants so inoculated to a healthy stock plant, where it was left for 3 days; then the plant was fumigated to kill the aphids.

As table 7 shows, no infections were obtained with any of the lots transferred from the fourth to the sixth day; the aphids had lost their infectivity after the first transfer. Infections were obtained with some lots transferred

on the seventh and later days, however; so that the aphids must have recovered the virus from the first inoculated plants. Breaking did not appear on these plants until 30 to 48 days after inoculation. Tompkins (1939) reported an incubation period of 16 to 22 days on mechanically inoculated plants. The virus was not recovered from 6 plants (not included in table 7) during the entire period.

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Plate 1. Heavy population of turnip aphids, *Rhopalosiphum pseudobrassicae*, on annual stock. White specks are molted skins. (Davis, California, August 3, 1934.)

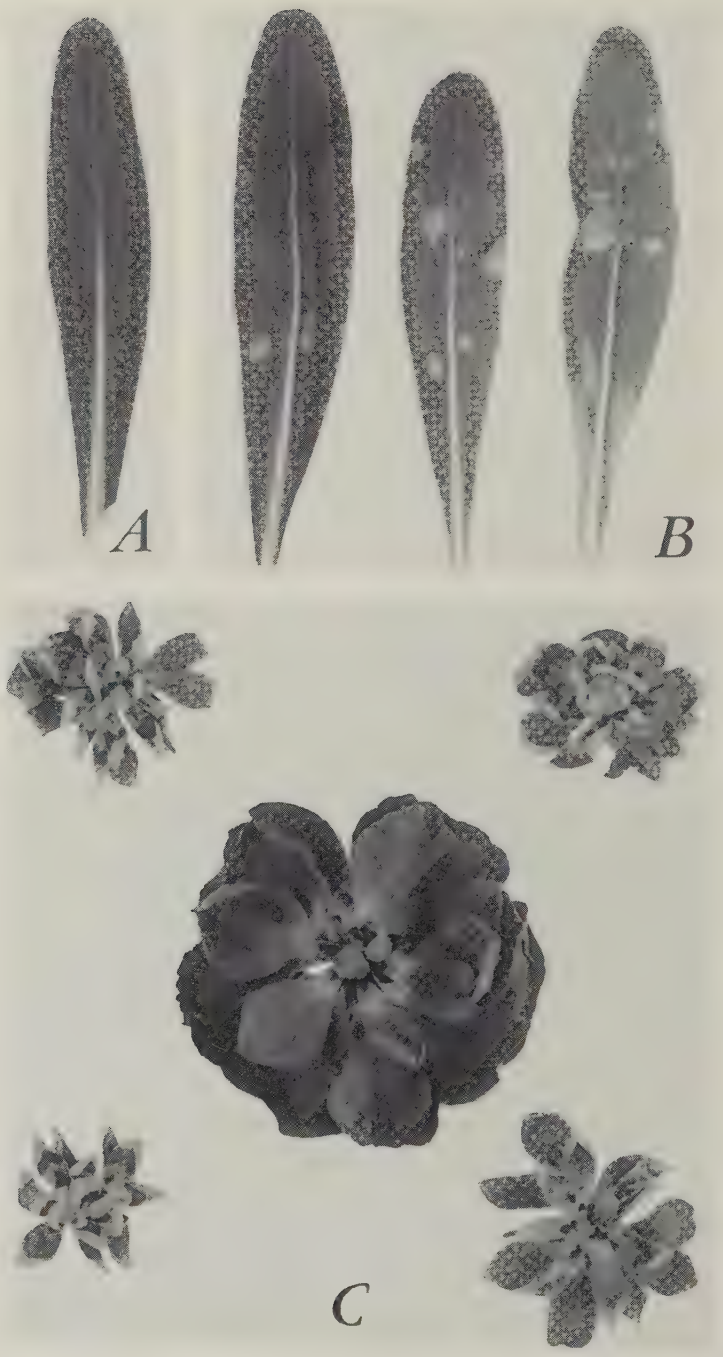

Plate 2. Symptoms produced by feeding of noninfective turnip aphid, *Rhopalosiphum pseudobrassicae,* on annual stock, *Mathiola incana* var. *annua: A,* leaf from healthy check or control plant on which no aphids had fed; *B,* pale-green circular areas around mouth-part punctures; *C,* center, normal flower of the Fiery Blood Red variety; grouped around it are four dwarfed flowers with tips of petals yellow or white or with all petals yellowish green.

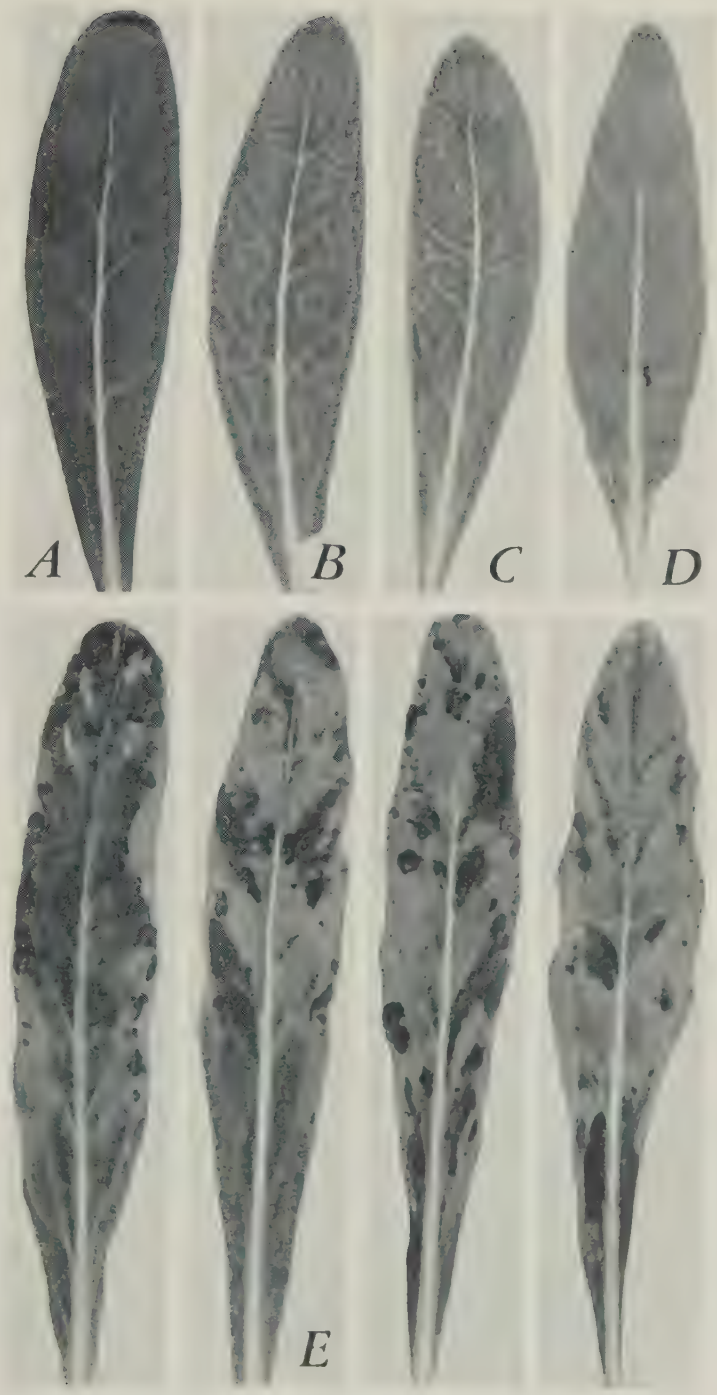

Plate 3. Symptoms produced by mild-mosaic virus on young leaves of annual stock, *Mathiola incana* var. *annua: A,* leaf from healthy plant; *B, C, D,* stages in clearing of veins and veinlets; *E,* mottling consisting of irregular-shaped pale and dark-green areas on young leaves (from a plant infected by means of the turnip aphid).

Plate 4. Field symptoms induced by mild-mosaic virus on flowers of naturally infected annual stock, *Matthiola incana* var. *annua*; Fiery Blood Red variety. *A*, apical cluster of flowers from a healthy plant; *B*, apical cluster of flowers showing breaking in color of petals from a naturally infected plant; *C*, center, normal flower; grouped around it are four flowers showing breaking, consisting of white areas and normal color. (Montara, San Mateo County, California, August 20, 1934.)

ADDITIONAL VIRUS DISEASES OF SPINACH IN CALIFORNIA[1]

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SUMMARY

Spinach was demonstrated to be naturally infected in California with sugar-beet mosaic and two cucumber mosaics—western cucumber mosaic and celery calico. Nine varieties of spinach were experimentally infected with the two cucumber mosaic viruses, five with sugar-beet mosaic. The three viruses were recovered from naturally infected spinach and transferred by mechanical inoculation to White Spine cucumber (*Cucumis sativus*), Turkish tobacco (*Nicotiana tabacum*), *N. glutinosa,* or sugar beet (*Beta vulgaris*).

The symptoms of these three diseases on spinach are similar. Filamentous leaves occur only in western cucumber mosaic. But cleared venation, chlorotic spotting, blisterlike elevations, and necrosis occur in all three. The viruses can be distinguished by transferring them to sugar beets; on that host the symptoms are sufficiently distinctive to permit identification.

New Zealand spinach (*Tetragonia expansa*) was experimentally infected with western cucumber mosaic, and the virus was recovered and transferred to White Spine cucumber and sugar beet.

The green peach aphid, *Myzus persicae* (Sulzer), is the most important vector of the western-cucumber-mosaic, celery-calico, and sugar-beet-mosaic viruses to spinach under natural conditions. The bean or dock aphid, *Aphis rumicis* Linnaeus, rarely transmits these viruses.

INTRODUCTION

Five virus diseases have been reported to occur on spinach (*Spinacia oleracea*, family Chenopodiaceae) under natural conditions in California; namely, aster yellows (Severin, 1934; Severin and Frazier, 1945),[3] sugar-beet curly top (Severin and Henderson, 1928; Scott, 1935), sugar-beet mosaic (Severin and Drake, 1948), spinach yellow dwarf (Severin and Little, 1947), and spotted wilt (Gardner, Tompkins, and Thomas, 1937)

This paper deals with two additional naturally occurring virus diseases of spinach—western cucumber mosaic and celery calico—and with sugar-beet mosaic on this host. Studies were made of the succession of symptoms on naturally and experimentally infected spinach caused by the three viruses. The symptoms of western cucumber mosaic were also studied on New Zealand spinach, *Tetragonia expansa*, which belongs to the family Aizoaceae. No insect-transmission tests are reported here. But previous reports on aphid transmission and on the aphid species naturally occurring on spinach are reviewed to determine which species are important vectors of the viruses.

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[3] See "Literature Cited" for citations, referred to in the text by author and date.

WESTERN CUCUMBER MOSAIC

Western cucumber mosaic occurs on several hosts in interior regions of California, but has not been found to occur naturally outside this region.

Outbreaks. During the spring of 1947, spinach in one 30-acre field near Patterson, in the northern San Joaquin Valley, was badly affected with yellowing of the foliage and rotting of the heart leaves. At first the injury was attributed to killing of the heart leaves by frosts. There was no evidence, however, of similar symptoms on spinach in other fields in the same district.

Later Bacon and Walz (1947) demonstrated that the rotting of the heart leaves resulted from the injuries of the seed-corn maggot, *Hylemya cilicrura* (Rondani), which also destroyed the young crown leaves of many plants.

Yellowing of the foliage, however, was present on many plants that showed no evidence of injury by the seed-corn maggot. On such plants the leaves showed large, blisterlike elevations (plate 1, *A, B*); some were malformed, thick, and leathery (plate 1, *B, C*). Since these symptoms suggested a virus disease, attempts were made to recover a virus. The western-cucumber-mosaic virus was recovered from spinach plants with yellow foliage, whether they showed injury from seed-corn maggot or not; it was transferred by the carborundum method (Rawlins and Tompkins, 1936) to sugar beets, which developed the typical symptoms (Severin and Freitag, 1948).

During the spring of 1948, a serious outbreak of western cucumber mosaic occurred on spinach in many fields near Westley and Patterson. The spinach turned yellow in one 40-acre field planted in September and was disked up in December. The 40 acres were replanted and again the spinach turned yellow and again was disked up. The virus was recovered from the second planting and transferred to sugar beets and squash. Spinach was demonstrated to be naturally infected with western cucumber mosaic in five other fields.

Symptoms. A noticeable symptom on naturally infected spinach, when the fields were viewed from a distance, was the yellow color of the entire plants, or yellow outer and green inner leaves; numerous dried or dead plants could be seen. A closer examination of some infected plants showed cleared veinlets on the youngest leaves (plate 2, *A*); and on intermediate leaves, circular chlorotic areas (plate 2, *C*), yellow blotches (plate 2, *B*), and necrotic areas in the yellow blotches (plate 2, *D*). Blisterlike elevations and malformations occurred on the heart leaves (plate 3, *A, B*). In the late stage of the disease, the youngest leaves are misshapen (plate 3, *D,* upper row) and frequently surround filamentous leaves (plate 3, *D,* lower row). When infected plants develop seedstalks, the filamentous leaves are very conspicuous (plate 3, *C*). In 1947 the blisterlike elevations were the prevailing symptom; in 1948 the filamentous leaves were prevalent and the blisterlike elevations rare.

The first symptom to appear on the youngest leaves of experimentally infected Bloomsdale (or Savoy-leafed) spinach is a clearing of the veins and veinlets (plate 4, *A*), accompanied with small, circular, chlorotic spots (plate 4, *A, B*). The younger leaves become malformed with blisterlike elevations (plate 4, *C, D*). Sometimes the younger leaves are folded along the midrib. In the late stage of the disease the newly developing leaves of some infected plants are linear, with blades reduced to but little more than the midrib.

Susceptible Varieties. The following nine varieties of spinach were experimentally infected with western cucumber mosaic: Bloomsdale or Savoy-leafed, Long Standing Bloomsdale, Broad Flanders, Giant Thick-leafed Nobel, Juliana, King of Denmark, Prickly Seeded, Virginia Savoy, and Viroflay.

The virus was recovered from each variety and transferred by mechanical inoculation to White Spine cucumber and to sugar beets.

New Zealand Spinach. The symptoms of western cucumber mosaic which appear on the leaves of New Zealand spinach, *Tetragonia expansa,* are wide yellow or pale-orange rings surrounding green tissue (plate 5, *A*). Later the green areas become chlorotic (plate 5, *B*) and often fuse (plate 5, *C*). The leaves frequently become malformed (plate 5, *D*). The yellow or orange rings may become necrotic. Necrosis of the margin of the leaf occurs. Later the entire leaf becomes dry.

The virus was recovered and transferred by mechanical inoculation to White Spine cucumber.

CELERY CALICO

Celery calico is a cucumber-mosaic virus which is common in the coastal fog belt and also occurs in the hot interior regions of California.

Natural Infection. The virus of celery calico was recovered from naturally infected spinach plants collected in vegetable fields near San Pablo. The virus extract was mechanically inoculated into healthy Long Standing Bloomsdale spinach, White Spine cucumber (*Cucumis sativus*), Turkish tobacco (*Nicotiana tabacum*), and *N. glutinosa;* typical symptoms of celery calico developed.

Symptoms. The older leaves of naturally infected spinach are lemon yellow (fig. 1, *A*). The younger leaves are bunched, narrowed, and deep green, with blisterlike elevations.

The first symptom to appear on the youngest leaves of inoculated varieties of spinach is a clearing of the veins and veinlets (plate 6, *A*) surrounding green areas (plate 6, *B*). The next symptom on the youngest leaves is the appearance of blisterlike elevations (plate 6, *C*); and the youngest developing leaves are narrowed and cupped downward. Later, necrosis of the younger leaves occurs. The oldest leaves become orange or lemon yellow (plate 6, *D*).

These symptoms are so similar to those of western cucumber mosaic (page 554) and sugar-beet mosaic (page 557) on spinach that a field infection of a spinach mosaic disease cannot be certainly identified by the symptoms on that host. It can be identified by transferring the virus to sugar beets; for on that host the symptoms are distinguishable (Severin, 1948).

Susceptible Varieties. The nine varieties of spinach infected with western cucumber mosaic were also experimentally infected with celery calico. The virus was recovered and transferred by mechanical inoculation to White Spine cucumbers, Turkish tobacco, and sugar beets.

SUGAR-BEET MOSAIC

Natural Infection. Spinach was demonstrated to be naturally infected with sugar-beet mosaic in vegetable gardens near San Pablo. The virus extract from diseased spinach collected in the field was inoculated in healthy spinach and sugar-beet plants, and typical symptoms of the disease developed.

Fig. 1. *A*, Symptoms of celery calico on a naturally infected plant of Long Standing Bloomsdale spinach, showing older leaves lemon yellow, and younger leaves bunched, narrowed, and deep green, with blisterlike elevations. *B*, Symptoms of sugar-beet mosaic on a plant of Long Standing Bloomsdale spinach, showing small chlorotic areas and cleared veinlets.

Symptoms. The symptoms on experimentally infected Giant Thick-leafed Nobel, Long Standing Bloomsdale, Prickly Seeded, Virginia Savoy, and Viroflay spinach are similar in most respects. The first symptom to appear is small, chlorotic areas on or between the veinlets and usually near the base of the youngest leaves (plate 7, *A*). These occur simultaneously with or are followed immediately by a broken type of cleared veinlets (fig. 1, *B*); in the greenhouse these cleared veinlets appear 10 days after inoculation. Numerous chlorotic rings develop, each with a necrotic center (plate 7, *B*). The young leaves assume a horizontal position (fig. 1, *B*). Later the chlorotic rings coalesce to form irregular chlorotic areas intermingled with conspicuous, dark-green, blisterlike elevations (plate 7, *C*), sometimes followed by chlorotic veinbanding (plate 7, *D*).

Contrary to Hoggan's (1933) observations, malformation on the youngest leaves is common on infected spinach here. Some dwarfed misshapen leaves show chlorotic areas and others blisterlike elevations (plate 8, *A*, *B*); others are twisted along the midribs (plate 8, *C*) or folded along the midribs (plate 8, *D*). Some of the youngest leaves are cupped outward, others are asymmetrical.

As the disease progresses, the older leaves show chlorotic rings which usually coalesce to form large, irregular, chlorotic areas interspersed with dark-green blotches (plate 8, *E*). Sometimes the chlorotic areas are smaller and more numerous, and the dark-green areas appear blisterlike, as on the younger leaves.

In the advanced stage of the disease, the older leaves usually show large, irregular, diffuse, yellowish areas which later become dark yellow or orange. Long Standing Bloomsdale and Virginia Savoy spinach developed numerous chlorotic rings, sometimes lenticular in shape, and measuring 4 mm in diameter, which later become necrotic. On all varieties the older leaves usually develop necrotic tissue at the tip; the necrosis gradually advances toward the base of the leaves; often it occurs along the margin or within the blade (plate 8, *F*). Necrosis gradually spreads over the older leaves and then toward the heart leaves. The dead tissue is papery, brown in color, and suggestive of sunburn. The plant finally dies.

Since spinach was so severely affected by the sugar-beet-mosaic virus, it may be of greater economic importance than is now realized.

Incubation Period. The incubation period of the disease in five varieties of spinach experimentally infected with sugar-beet mosaic ranged from 7 to 15 days. The number of times that the virus was recovered has been reported in a previous paper (Severin and Drake, 1948).

APHID VECTORS

Patch (1938) lists the following species of aphids as occurring on spinach under natural conditions.

Cotton or melon aphid, *Aphis gossypii* Glover
Bean or dock aphid, *Aphis rumicis* Linnaeus
Hyalopterus atriplicis Linnaeus
Potato aphid, *Macrosiphum solanifolii* Ashmead
Green peach aphid, *Myzus persicae* (Sulzer)

Bacon and Walz (1947), who carried on extensive tests on the aphid populations on spinach in the San Joaquin, Santa Clara, and Salinas valleys during the springs of 1946 and 1947, found that the green peach aphid, *Myzus persicae,* was the most abundant species on spinach. The bean or dock aphid, *Aphis rumicis,* was occasionally taken on spinach, and some of the females had given rise to small colonies of aphids. Winged forms of the pea aphid, *Macrosiphum pisi* (Kaltenbach), were observed on spinach but were not multiplying. Previous tests (Severin, 1942; Severin and Freitag, 1948; Severin and Drake, 1948) have shown that the green peach aphid transmits the western-cucumber-mosaic, sugar-beet, and celery-calico viruses; and that the bean aphid rarely transmits the first two. Hence, and also because of its abundance on spinach, the green peach aphid is the most important vector of these viruses to spinach, under natural conditions. The other species of aphids listed by Patch as occurring on spinach were not tested.

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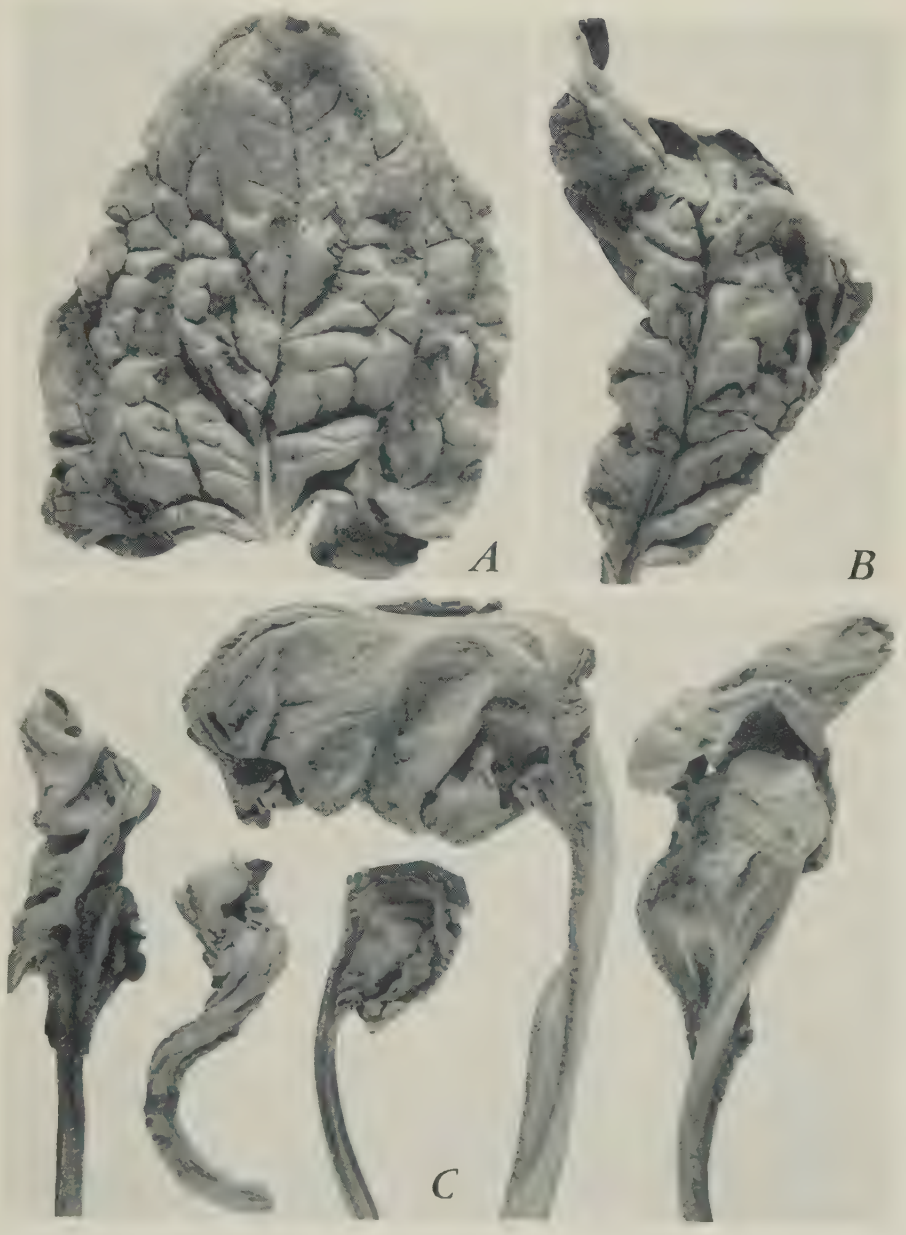

Plate 1. Symptoms of western cucumber mosaic on leaves of naturally infected spinach, *Spinacia oleracea: A*, blisterlike elevations; *B*, malformed and blistered younger leaf; *D*, misshapen leaves.

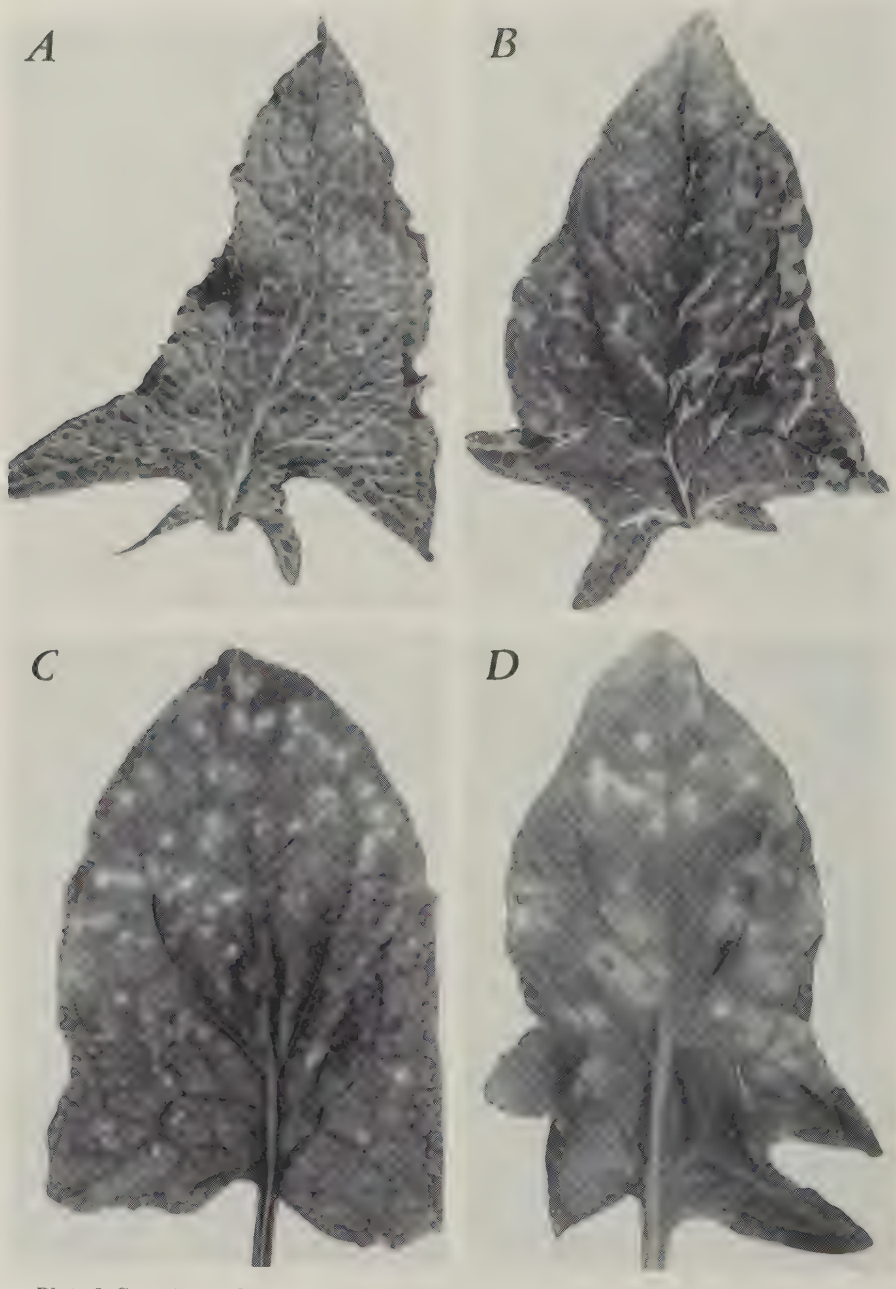

Plate 2. Symptoms of western cucumber mosaic on leaves of naturally infected spinach: *A*, cleared veinlets on youngest leaf; *B*, yellow blotches; *C*, circular chlorotic areas; *D*, necrotic areas in yellow blotches, on intermediate leaf. These symptoms and those shown in plate 1 cannot be distinguished with certainty from corresponding symptoms produced by celery calico and sugar-beet mosaic on spinach.

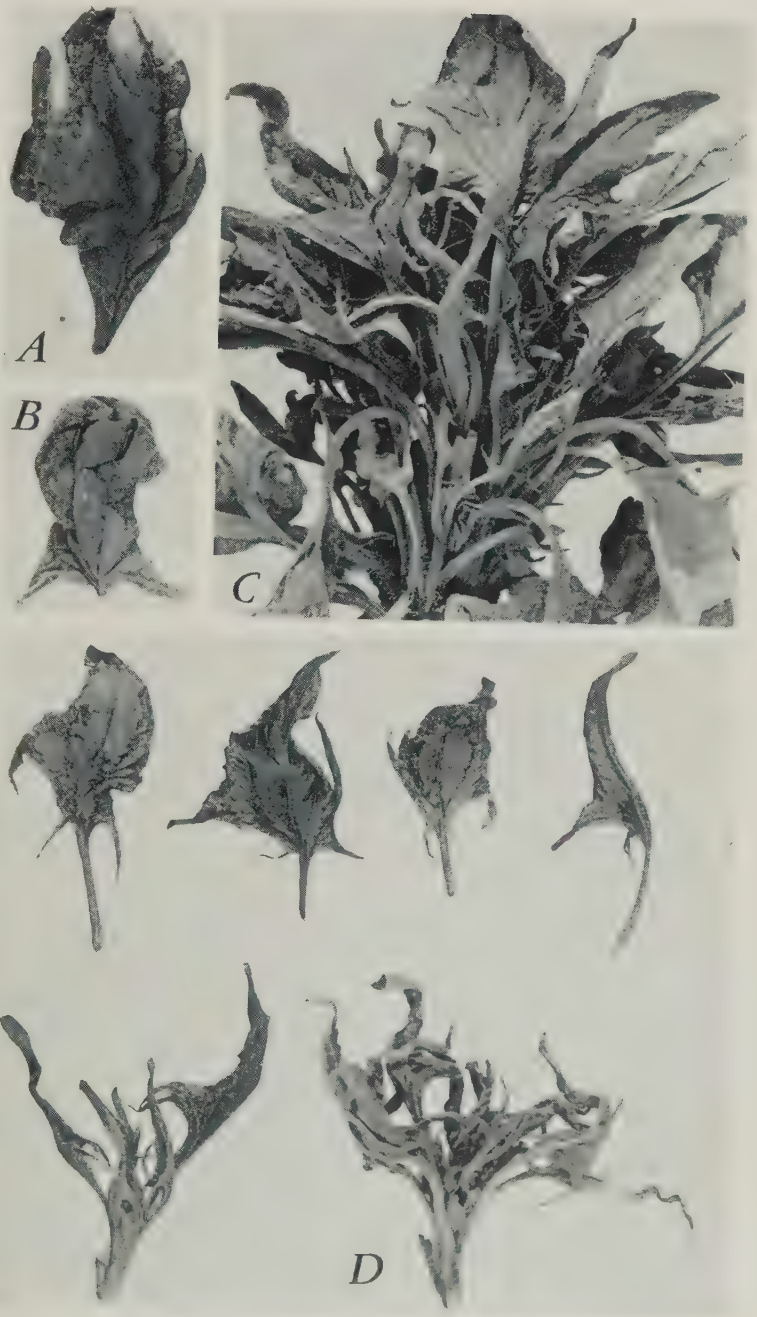

Plate 3. Symptoms of western cucumber mosaic on naturally infected spinach: *A*, *B*, blisterlike elevations and malformations on heart leaves; *C*, malformed and filamentous leaves growing from seedstalk; *D*, upper row, misshapen leaves; *D*, lower row, malformed leaves surrounding filamentous leaves.

Plate 4. Symptoms of western cucumber mosaic on leaves of experimentally infected Bloomsdale spinach: *A*, cleared veins and veinlets accompanied by small, circular, chlorotic spots on youngest leaf; *B*, narrowed leaf showing numerous chlorotic spots, some fusing; *C*, *D*, malformed leaves with blisterlike elevations and apical chlorosis.

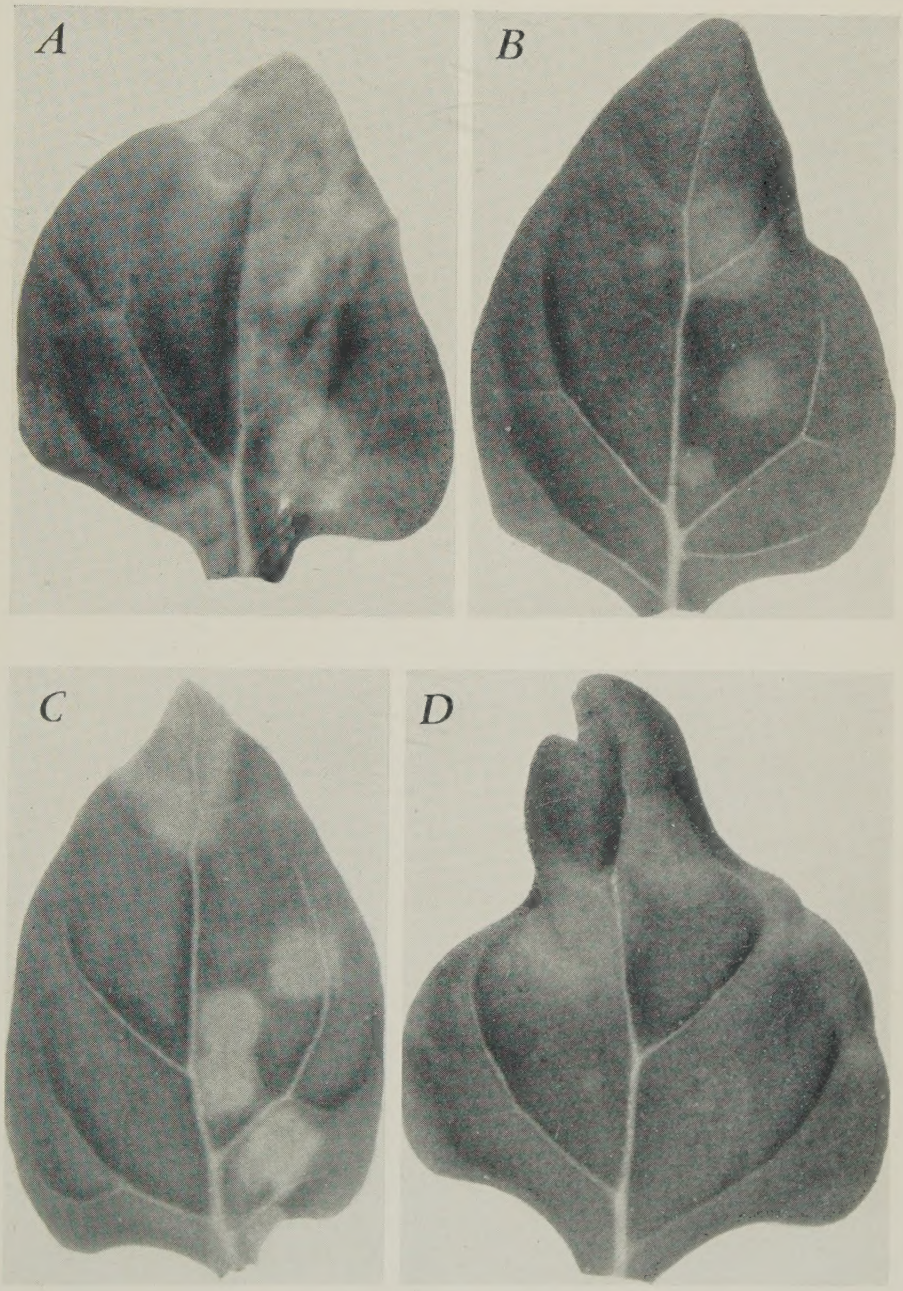

Plate 5. Symptoms of western cucumber mosaic on leaves of experimentally infected New Zealand spinach, *Tetragonia expansa* (family Aizoaceae): *A*, wide yellow or orange rings which surround green tissue; *B*, circular chlorotic areas; *C*, circular chlorotic areas fusing; *D*, malformed leaf with chlorotic areas. Blisterlike elevations and filamentous leaves, such as occur on spinach, have not been observed on this species.

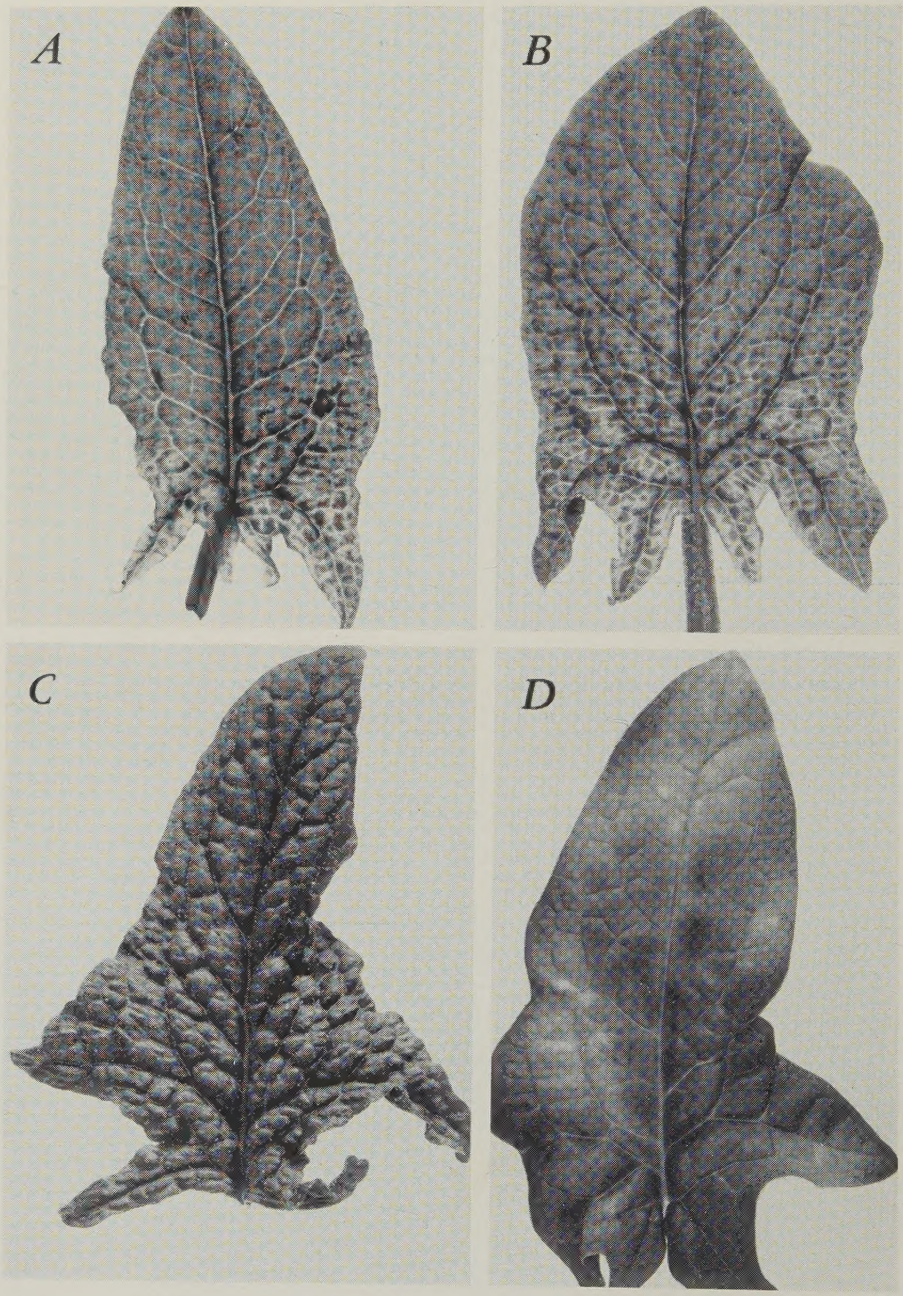

Plate 6. Symptoms of celery calico on leaves of experimentally infected Long Standing Bloomsdale spinach: *A, B,* youngest leaf, showing cleared veins and veinlets, surrounding green areas in *B; C,* blisterlike elevations; *D,* oldest leaf showing orange or lemon-yellow discoloration.

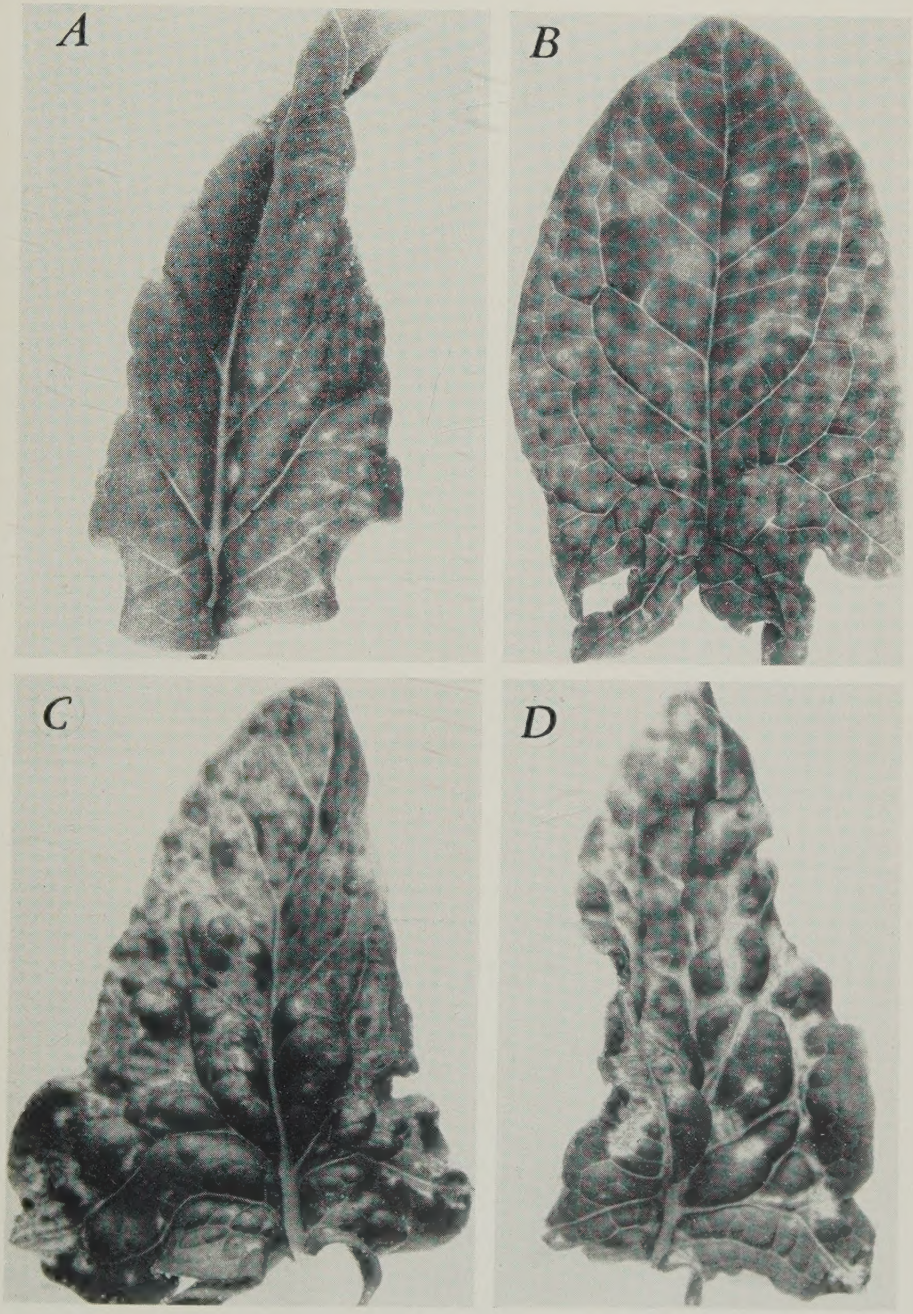

Plate 7. Symptoms of sugar-beet mosaic on young leaves of Long Standing Bloomsdale spinach: *A*, small chlorotic areas on and between the veins; *B*, small, circular, chlorotic rings, each with a minute necrotic center; *C*, chlorosis and blisterlike elevations; *D*, blisterlike elevations and veinbanding.

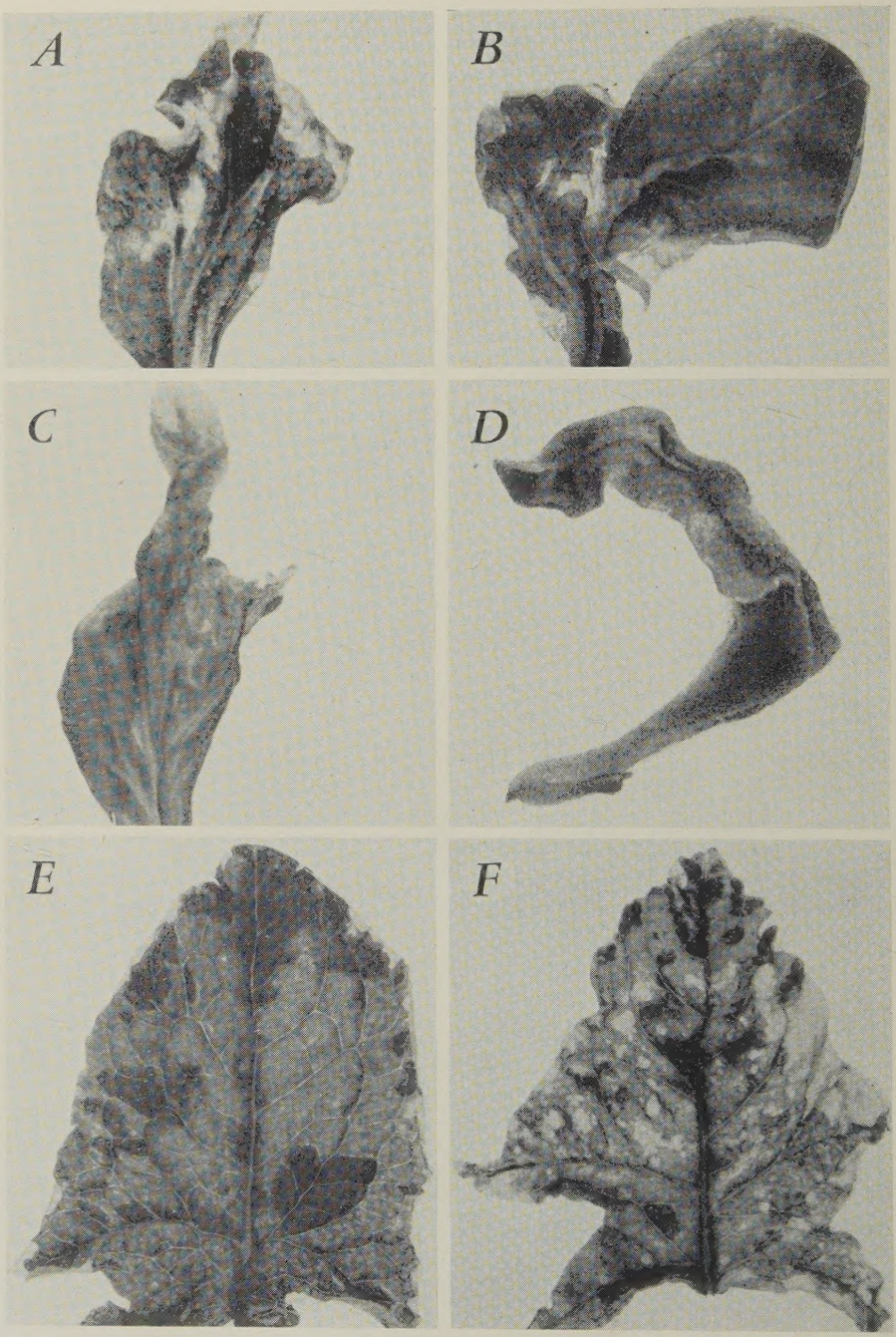

Plate 8. Symptoms of sugar-beet mosaic on leaves of Long Standing Bloomsdale spinach: *A*, *B*, dwarfed, malformed, young leaves showing blisterlike elevations; *C*, young leaf twisted along the midrib; *D*, young leaf folded along the midrib; *E*, old leaf showing large, irregular, chlorotic areas and dark-green blotches; *F*, old leaf showing necrosis along the margin and within the blade.